Chapter 70
Opposite Roles of MerTK Ligands Gas6 and Protein S During Retinal Phagocytosis

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Abstract MerTK is required for photoreceptor outer segment (POS) phagocytosis by retinal pigment epithelial (RPE) cells, a diurnal function essential for vision maintenance. In vivo, MerTK is stimulated at the time of the phagocytic peak through an intracellular signaling pathway. However, MerTK ligands Gas6 and Protein S are expressed in both RPE cells and photoreceptors, and at least one of them required for phagocytosis to occur. Still, their exact role in the retina was not clear until recently. This review combines results from different studies to shed the light on a tissue-specific regulation of MerTK function by its ligands. Indeed, with opposite effects on RPE phagocytosis and changes in their expression levels around the time of POS uptake, Gas6 and Protein S may contribute to the tight control of the acute phagocytic peak in the retina.

Keywords Retinal pigment epithelium · Phagocytosis · Photoreceptor outer segments · MerTK · Ligands · Gas6 · Protein S · Circadian rhythm

70.1 Introduction

MerTK is the internalization receptor necessary for phagocytosis of apoptotic cells by macrophages (Scott et al. 2001), as well as of photoreceptor outer segments (POS) by RPE cells (D'Cruz et al. 2000; Nandrot et al. 2000; Feng et al. 2003). In the retina, this task allows the elimination of aged POS tips on a daily basis following a circadian rhythm peaking 2 h after light onset (Young and Bok 1969; LaVail 1976). Phagocytosis is crucial to alleviate the oxidative stress linked to constant light exposure of photoreceptors and to allow the renewal of POS membranes (Strauss 2005). When deregulated or absent, retinal pathologies ensue with an either early or late onset – such as rod-cone dystrophies with early macular involvement

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(atypical retinitis pigmentosa) or age-related macular degeneration (AMD) – in animal models and patients (Dowling and Sidman 1962; Gal et al. 2000; Tschernutter et al. 2006; Nandrot et al. 2004).

Until recently, the role of MerTK ligands in the retina was not clear because MerTK is directly activated in vivo at the time of the phagocytic peak via an intracellular signaling pathway initiated by the alphavbeta5 integrin–Mfg-E8 receptor–ligand couple (Nandrot et al. 2004; Nandrot et al. 2007). In addition, single-knockout models for each ligand do not develop any phenotype (Hall et al. 2005; Prasad et al. 2006; Burstyn-Cohen et al. 2012), while RCS rats lacking MerTK are affected by a severe retinal degeneration rendering them blind by the age of 3 months (Dowling and Sidman 1962). The interest in studying MerTK ligands during POS phagocytosis was sparked again when a study showed that double-knockout mice present a degenerative phenotype similar to RCS rats (Burstyn-Cohen et al. 2012).

70.2 Presentation of MerTK and Related Ligands Gas6 and Protein S

70.2.1 The TAM Receptor Family and Apoptotic Cell Recognition

MerTK belongs to the TAM – Tyro3, Axl, and MerTK – family of tyrosine kinase receptors (Hafizi and Dahlbäck 2006a). TAM receptors are composed of an extracellular domain – two immunoglobulin-like domains and two type III fibronectin repeats –, a transmembrane domain and a cytoplasmic domain mainly comprising the tyrosine kinase domain. TAMs are not necessary during development but carry a role in general tissue homeostasis through the elimination of various kinds of apoptotic cells (AC) and the control of innate immune system responses (Lemke 2013). However, in the retina only MerTK and Tyro3 are expressed in RPE microvilli, and absence of Tyro3 does not lead to any retinal degeneration (Prasad et al. 2006).

Specific recognition of ACs and POS extremities requires exposure of phosphatidylserines (PtdSer) on their surface (Fadok et al. 1992; Ruggiero et al. 2012). PtdSer are normally found on the inner leaflet of the plasma membrane but flip to the outer leaflet when cells become apoptotic for immediate recognition and clearance by macrophages. In the retina, only the outmost tip of POS to be tethered and engulfed by RPE cells exposes PtdSer in a timely fashion (Ruggiero et al. 2012). PtdSer are recognized either directly by receptors on the phagocyte surface such as CD36 (Ryeom et al. 1996) or indirectly via bridge molecules such as Mfg-E8 and Gas6/Protein S (Hanayama et al. 2002; Nandrot et al. 2007; Nakano et al. 1997; Anderson et al. 2003; Hall et al. 2002).

70.2.2 Gas6 and Protein S: Two Shared Ligands with Similar Structures

Two cognate ligands have been described for TAM receptors, Gas6 and Protein S (Stitt et al. 1995; Varnum et al. 1995). These vitamin K-dependent ligands share a similar molecular structure: a Gla domain (PtdSer binding), four EGF-like domains, and a sex-hormone-binding globulin domain (receptor binding) (Hafizi and Dahlbäck 2006b). However, they can be used in distinct functions. Protein S has a prominent role in the anticoagulation cascade and has been shown to be implicated in atherosclerosis and angiogenesis though its participation in phagocytosis (Walker 1980; Liao et al. 2009; Burstyn-Cohen et al. 2009). The Gas6–Axl complex has also been linked to angiogenesis inhibition (Gallicchio et al. 2005) and is in general associated with cell survival and/or proliferation (Melaragno et al. 2004; Stenhoff et al. 2004), as well as cell migration and adhesion (McCloskey et al. 1997).

Importantly, Gas6 and Protein S are implicated in AC clearance by macrophages (Nakano et al. 1997; Anderson et al. 2003). First linked to PtdSer, Protein S molecules bind to TAM receptors and mediate their activation by inducing receptor dimerization and autophosphorylation (Uehara and Shacter 2008). Gas6 has the capability to bind PtdSer and stimulates receptor activation (Nakano et al. 1997; Hall et al. 2002), but its precise role in apoptotic cell clearance is not clear. Gas6 absence does not lead to any phenotype in mice, thus Protein S is sufficient to elicit phagocytosis (Prasad et al. 2006; Lew et al. 2014).

70.2.3 What About the Retina?

Since the identification of MerTK in RCS rats, studies have been testing the implication of Gas6 and Protein S in POS elimination. In vitro, both have been shown to stimulate POS phagocytosis by primary RPE cells and retinal explants, with a prominent role for Protein S (Hall et al. 2001, 2002, 2005; Prasad et al. 2006). Surprisingly, Gas6 and Protein S are both expressed by the retina and RPE cells (Hall et al. 2001; Prasad et al. 2006). This raises the following question: do both RPE and photoreceptors contribute to POS phagocytosis?

As well, in absence of a phenotype in single-knockout mice, their in vivo contribution was debated, until the creation of a mouse model inactivated for both Gas6 and Protein S that develops a blindness similarly to MerTK-deficient rats or mice (Burstyn-Cohen et al. 2012). This phenotype made it clear that at least one of these ligands is required in the retina, potentially because of their role in MerTK dimerization itself necessary for further intracellular receptor activation (Uehara and Shacter 2008; Lew et al. 2014). However, no other participation in the phagocytic process had been investigated.

70.3 Role of MerTK Ligands During Retinal Phagocytosis

70.3.1 Opposite Roles During in Vitro Phagocytosis

We recently investigated the effect of increasing doses of Gas6 and Protein S, either alone or in combination, on POS phagocytosis by RPE-J cells in comparison with J774 macrophages (Law et al. 2015). As expected, both ligands stimulated macrophages at all doses with an additive effect at some doses when combined. Surprisingly, increasing doses of Gas6 appear to inhibit RPE-J cell phagocytosis, while Protein S has a dose-dependent stimulatory effect, and they compensate each other when combined (Fig. 70.1). This suggests that, in the retina, changes in Gas6 and Protein S amounts could contribute to the regulation of MerTK activity.

70.3.2 In Vivo Variation of Expression Levels Along the Light/Dark Cycle

In order to assess respective levels of each ligand, we analyzed their mRNA expression profiles along the light/dark cycle by qPCR on isolated retina and RPE/choroid (RPE/Ch) (Parinot et al. under revision). Overall *Gas6* was more expressed than *Pros1* (Protein S), and more present in the retina than RPE/Ch as previously suggested (Hall et al. 2005). *Gas6* expression in RPE/Ch decreased just before the phagocytic peak and increased just after (Fig. 70.2), while in the retina it was quite stable with a slight increase just before the peak (data not shown). *Pros1* expression followed a bimodal rhythm: it increased markedly just before and at phagocytic

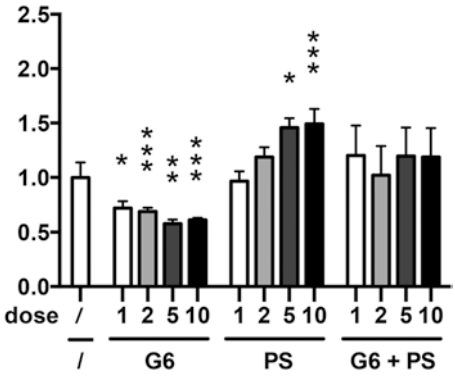

Fig. 70.1 Antagonistic role of MerTK ligands Gas6 and Protein S on POS phagocytosis by RPE-J cells. POS internalization (FITC/DAPI ratio) was quantified after cells were challenged for 3 h with FITC-POS alone (/) or with the addition of various doses of Gas6 (G6) and/or Protein S (PS) as indicated (μg/mL). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Modified from © Law et al. 2015. Originally published in *The Journal of Biological Chemistry*, https://doi.org/10.1074/jbc.M114.628297)

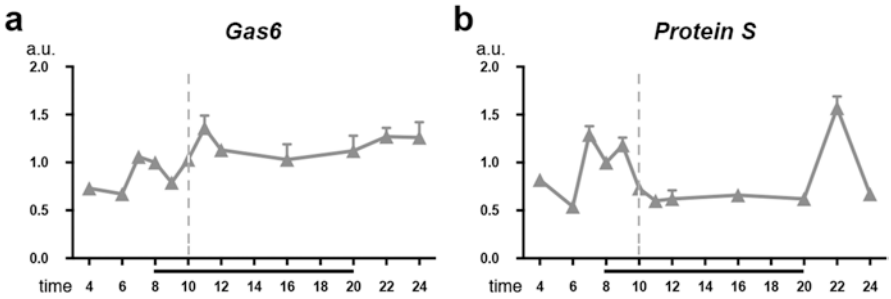

Fig. 70.2 Different cyclic expression of MerTK ligands *Gas6* and *Pros1* mRNAs. mRNA expression profiles for *Gas6* (**a**) and *Pros1* (**b**) in the RPE/choroid of wild-type animals at different times of the day as indicated were assessed by qPCR. The black bar under each graph represents the time points during which lights are on in the animal facility, and the dotted vertical line indicates phagocytic peak time. All quantifications are expressed in arbitrary units (a.u.) as mean ± SD, $N = 3–6$ independent samples. The reference set as 1 for comparison is the quantification at 8:00 AM (light onset) for each gene (From Parinot et al. under revision)

peak time in RPE/Ch (Fig. 70.2) and retina, respectively, and a second peak occurs at light offset (retina) and right after (RPE/Ch). This suggests that higher amounts of stimulatory Protein S may be available in the interphotoreceptor matrix (IPM) in time for POS phagocytosis while inhibitory Gas6 levels decrease.

70.4 Perspectives

Activity of MerTK can be regulated by many ways mostly through intracellular activation via alphavbeta5 integrin signaling (Nandrot et al. 2004) and by the cleavage of its extracellular domain both in vitro and in vivo (Law et al. 2015). This cleavage is augmented by Gas6 and diminished by Protein S, which could explain their respective inhibitory and stimulatory roles on RPE phagocytosis. Thus, bioavailable levels of each ligand in the IPM at different times of the light/dark cycle might help control the height and the duration of MerTK activation, hence contributing on a daily basis to the tight control of the sharp peak of POS phagocytosis. In addition, these results suggest a potential competition for ligand binding on MerTK.

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